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PETIOLE ANATOMY AND MINOR VEINS AS AIDS FOR THE IDENTIFICATION OF *SYMPLOCOS* OF NEPAL

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ABSTRACT

On a study of eleven species of *Symplocos* occurring in Nepal, it is observed that petiole anatomy and minor venation pattern are helpful for identifying sterile material. Two species, *S. pyrifolia* and *S. sumantia* have exactly similar characters but can be distinguished from the other nine species. Sclerenchymatous bundle sheaths are known in a few dicotyledonous families like Winteraceae and Melastomataceae, and in a few genera of Dilleniaceae. It is significant that such sclerenchymatous bundle sheaths are also found in some species of *Symplocos*. It is felt that extensive survey of all the species of *Symplocos* would prove to be very interesting.

INTRODUCTION

Leaf anatomy has been widely employed in taxonomically difficult groups like Cruciferae and Graminae. Carlquist (1957a, 1957b) has made use of anatomical information as an aid for clarifying the systematic relationships of some Composits, and according to Hagerup (1953), the vegetative organs of the Ericales, particularly the leaves are most remarkably useful in taxonomy. Lems (1964), on a study of leaf and petiole sections and cleared leaves of various species of Andromedeae, showed that the North American species may be identified by their leaf characteristics.

According to Howard (1962), the vascular structure of the petioles appear to be most useful at the generic level. Certain families show a variety of patterns indicating that the petiole vascular anatomy can be profitably used in descriptions and indentifications. Howard (*loc. cit.*) has found that in a few cases the petiole vascular pattern is useful at the specific level; however, in most cases striking differences appear to support generic segregation. Further, the petiole vascularisation of 20 genera of Luxemburghieae (Ochnaceae) have revealed variations which appear to Decker (1967) significant in determining the phylogeny of the tribe. Schofield (1968) has also found petiole anatomy to be a useful 'taxonomic tool'.

Briefly to trace the history of venation study, no one can do better than to quote Howard (1959 p. 171), "The foundations of the study of venation systems in the leaves of angiosperms were laid by Von Ettinghausen (1854a, 1854b, 1861, 1865). Through his prodigous efforts several magnificent monographs on venation patterns were published". There has been a resumption of interest in the study of venation patterns in recent years, due to the work of Goebel (1922), Troll (1938), Foster (1950a, 1950b, 1951) and others who have shed new light on the foliar vasculature of plants.

It has been in the mind of the author to search for suitable anatomical characters that could serve as an additional tool to the taxonomist in the study and determination of sterile specimens of *Symplocos*.

In Nepal, there are eleven species of *Symplocos*, most of which are found at altitudes of 1,525 to 3,200 m. *S. ramosissima* a common species occurring in association with *Quercus* species, along with species of *Castanopsis*, constitutes a prominent element of the temperate mixed broad-leaved forests.

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S. spicata is a common constituent of the subtropical evergreen or semievergreen forests, while *S. crataegoides* occurs in the coniferous forests at altitudes ranging from 2,135 m to 2,895 m. The majority of species, flower during the months of March to May; some flower during October and November and even in December. Certain species like *S. pyrifolia, S. sumantia, S. dryophila* and *S. phyllocalyx* are difficult to identify when the plants are not in flowers. Petiole anatomy, venation pattern, besides the veinlets terminations along the leaf margins, have been studied of eleven species occurring in Nepal to find out anatomical characters of taxonomic value.

MATERIALS AND METHODS

The study was carried out on material from the author's herbarium, and some material received from the Herbarium of Nepal Government (KATH.). The entire material for the present study was collected from Nepal.

Petioles were soaked in absolute alcohol for 24 hours and then gradually brought down to water. Later, the materials were transferred to 8% KOH solution and kept for 2-3 days depending on the thickness of the petioles. This made the materials to swell and soften. Next, the materials were washed in 15% glacial acetic acid; after several changes in water, they were gradually dehydrated. The petioles were embedded in paraffin following the customary procedure and sectioned at 14-16 microns. Sections were cut at the proximal, middle and the distal ends of the petioles. When the petiole was short, serial sections were cut.

For the study of vascularisation of the lamina or its segments of convenient size, they were first soaked in water and later transferred to 5% KOH for several days. The duration of the materials in KOH depended on the thickness of the leaf and its cuticle. After clearing in KOH, the materials were later transferred to Choral hydrate, following Arnott (1959), and finally stained in 1% aqueous Safranin and mounted in glycerine jelly. Slides in glycerine jelly, although temporary, were suitable for photomicrography.

OBSERVATIONS

Basing on the anatomical characters observed, a tentative key is proposed for the identification of the following eleven species of *Symplocos* occuring in Nepal:

- A Petiolar vascular bundle unbranched
  - B Bundle sheath of leaf veinlets sclerenchymatous
    - C Median vascular bundle of petiole V-shaped ; ratio between veins of 3rd category and last category 1.5 : 1 ; veinlets taper gradually and terminate in the marginal serrulations ... *caudata*
    - CC Median vascular bundle of petiole not V-shaped
      - D Median vascular bundle of the petiole crescent-shaped ; ratio between veins of 3rd and last category 8 :1; veinlets along margins of leaf broom-shaped ... *ramosissima*
      - DD Median vascular bundle of the petiole in the form of a flattened arc ; ratio between the veins of 3rd and last category 2.5 : 1 ; veinlets along margins of leaf broom-shaped ... *pyrifolia/sumantia*
  - BB Bundle sheaths of leaf veinlets parenchymatous
    - C Median vascular bundle of petiole in the form of a flattened arc
      - E Main veins very thick, ratio between veins of 3rd and last category 6 : 1 ... *racemosa*
      - EE Main veins slender, ratio between veins of 3rd category and last category c 2.5 : 1
        - F Bundle sheath very thin and faint ; veinlets along margin of leaf broom-shaped ... *phyllocalyx*
        - FF Bundle sheath not faint but clear : veinlets along margin of leaf taper gradually and terminate in the serrulations ... *theaefolia*
    - C C Median vascular bundle of the petiole crescent-shaped
      - G Terminal free endings of veinlets unbranched or ending in a few dichotomies (stag hornlike) ... *dryophila*
      - GG Terminal free endings or veinlets dichotomously branched ; marginal veinlets broom-shaped ... *glomerata*
- AA Petiolar vascular bundle giving branches
  - H Lateral branches arising from middle of the petiole ... *crataegoides*
  - HH Lateral branches arising from proximal end of petiole ... *laurina*

It will be seen from the above key that *S. pyrifolia* and *S. sumantia* have exactly the same characters and can not be distinguished between themselves but they are clearly distinguishable from the other nine species.

S. caudata Wall. ex G. Don, Gen. Syst. 4: 3. 1837; Clarke in Fl. Brit. Ind. 3: 577. 1882.

Taxonomy: Usually shrubs sometimes reaching 4.5 m in height. Leaves lanceolate-caudate, 4.5 × 1.8 cm, subentire, lamina base cuneate, chartaceous, petiole c 5 mm long. Flowers in lax racemes; bracts and bracteoles inconspicious, sparsely pilose; pedicels c 4 mm long, calyx tube obconic, teeth ovate, corolla c 3 mm long; stamens about 25. Fruit ovoid-cylindric, c 8 mm long, shining, crowned by the enlarged calyx-teeth.

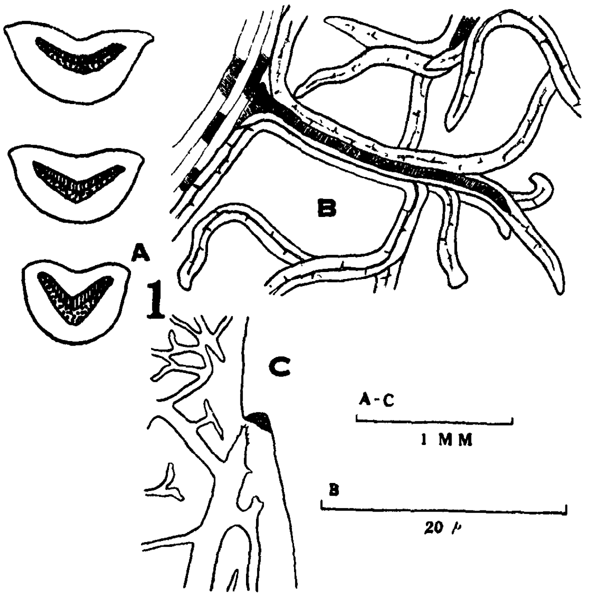


Fig. 1. *S. caudata*
(A-Trans. sections of the petiole; B-Vien endings with the bundle sheath; C-Marginal serration)

Anatomy: *Petiole*—The median vascular bundle is V-shaped and does not give off branches. It, however, becomes arc-shaped at the distal end (Fig. 1A). In the cortical region there is no sclerenchyma.

Venation: The ratio between the veins of the 3rd category and the last category is 1.5: 1 (Fig. 10). The bundle sheath is fibrous (Fig. 1B). The very tips of the free vein endings are occupied by tracheids which are elongate or clavate. Along the margin of the leaf, the veinlets taper gradually and terminate in the marginal serrulations (Fig. 1C).

S. crataegoides Buch.-Ham. ex D. Don, Prodr. Fl. Nep. 145. 1825; Clarke in Fl. Brit. Ind. 3: 537. 1882. *S. chinensis* (Lour.) Druce in Rep. Bot. Exch. Cl. Brit. Isles, 1916: 605. 1917. *Myrtus chinensis* Lour. Fl. Cochinch. 313. 1790.

Taxonomy: Trees c 12 m tall, sometimes shrubs. Leaves broadly elliptic, sometimes lanceolate, obovate-elliptic, c 6 × 3.75 cm, acuminate, sharply serrulate towards apex, but teeth obsolete lower down. Flowers in panicles, lateral branches cymosely many-flowered; bracts small, caducous; calyx-tube obconic, pilose or glabrous; corolla c 3 mm; stamens indefinite. Fruit c 3 mm long, obliquely ovoid; calyx-teeth small not enlarged in fruit.

Anatomy: *Petiole*—The median vascular bundle is crescent-shaped giving off small branches from the middle of the petiole. The number of branches is variable but usually one trace is given off on either side (Fig. 2A). There is no sclerenchyma in the cortical region.

Venation: The ratio between the veins of 3rd and last category is 3: 1 (Fig. 12). The bundle sheath is parenchymatous, the cells of which are regular in shape (Fig. 2B). The very tips of the free vein-endings are made up of tracheids which are elongate and clavate, and the terminations of the marginal veins are fusiform (Fig. 2C).

S. dryophila Clarke in Fl. Brit. Ind. 3: 578. 1882.

Taxonomy: Trees with glabrous branch-

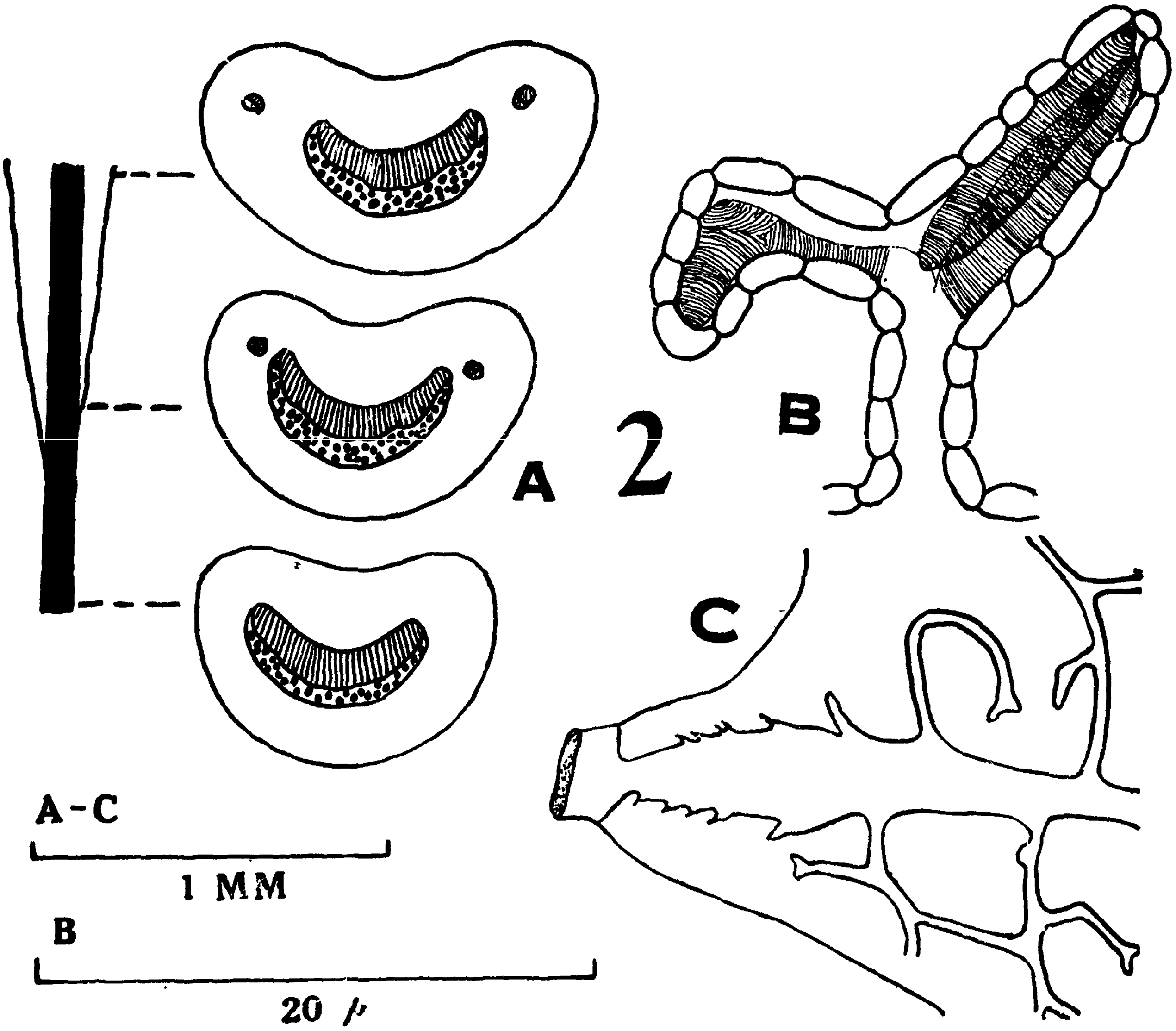


Fig. 2. *S. crataegoides*
(A-Trans. sections of the petiole; B-Vien endings with the bundle sheath; C-Marginal serration)

lets. Leaves obovate lanceolate, c 15 × 3.75 cm, lamina base cuneate, petiole c 8 mm long, subentire, densely villose when young. Flowers in lax racemes; bracts orbicular, caducous; calyx-tube c 2 mm, teeth minute; corolla c 4 mm; stamens 20-25. Fruits c 5 mm in diam., subglobose, crowned by very narrow calyx-rim.

Anatomy: *Petiole*—The median vascular bundle is crescent-shaped and near its distal end two branches are given on either side (Fig. 3).

Venation: The ratio between veins of 3rd and last category is 2: 1 (Fig. 14). The bundle sheath is parenchymatous. The very tips of the free vein-endings are unbranched or there may be a few dichotomies, and the terminal elements consist of clavate tracheids.

S. glomerata King ex Clarke in Fl. Brit. Ind. 3: 577. 1882.

Taxonomy: Trees c 3 m tall, sometimes 4.5 to 6 m, and at times shrubs. Leaves elongate-lanceolate, c 15 × 3.75 cm, acuminate, lamina base cuneate, glandular serrate,

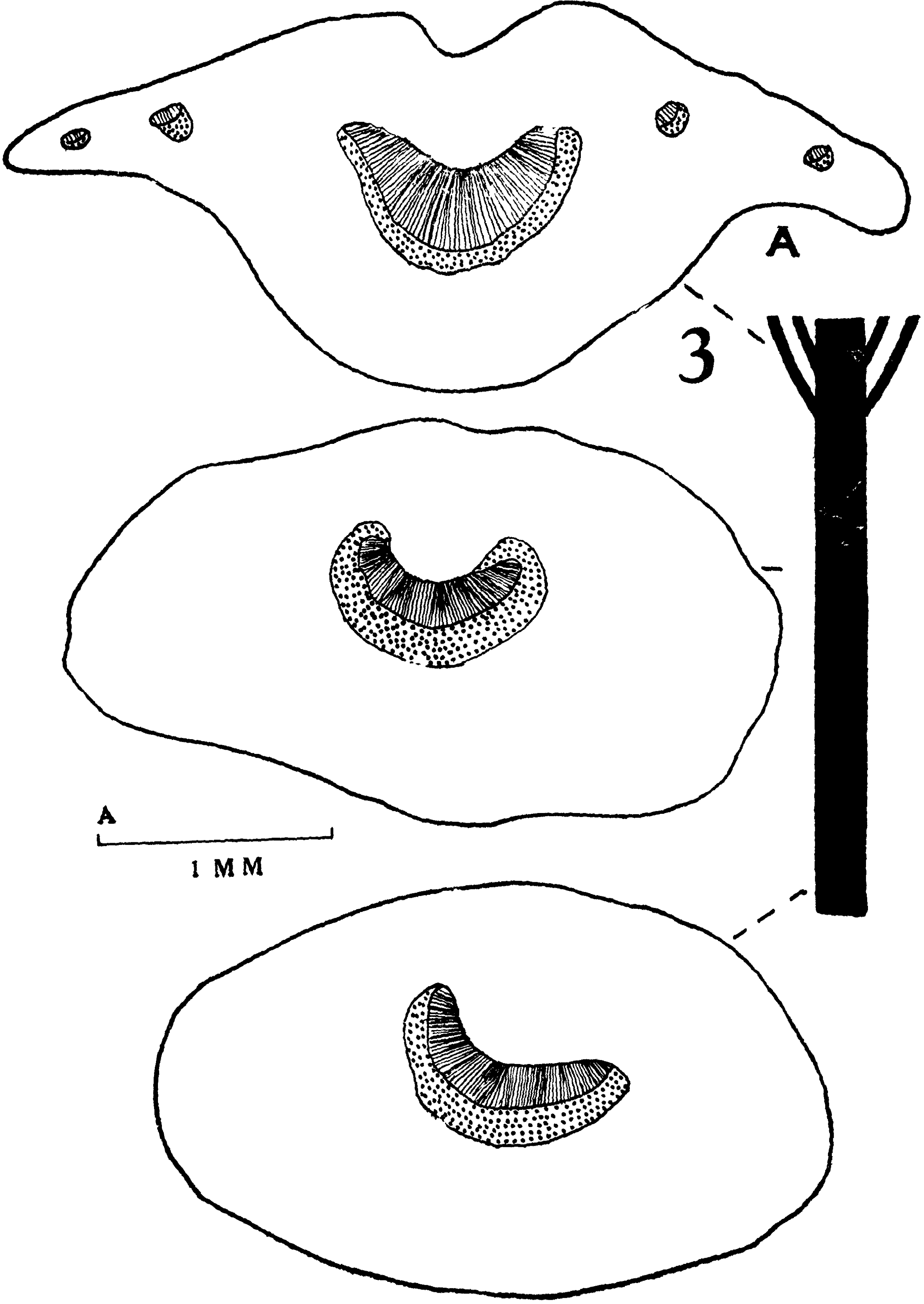


Fig. 3. *S. dryophila*
(A-Trans. sections of the petiole)

coriaceous; petiole c 1.2 cm long, glandular. Flowers densely fascicled; bracts ovate, obscure; calyx-tube broadly rounded, teeth c 1 mm, glabrous; corolla c 4 mm; stamens 20-25. Fruit c 6 mm long, subcylindric; calyx-rim as wide as the fruit with erect teeth.

Anatomy: *Petiole*—The median vascular bundle is crescent-shaped and does not give off any branch (Fig. 4A). In the cortical region some sclerenchyma may be present.

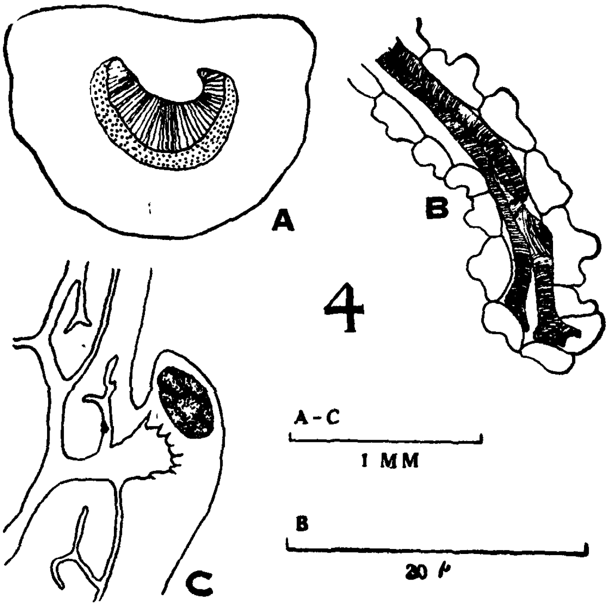


Fig. 4. *S. glomerata*
(A-Trans. sections of the petiole; B-Vein endings with the bundle sheath; C-Marginal serration)

Venation: The ratio between the veins of 3rd and last category is 3:1. The bundle sheath is of irregular parenchymatous cells (Fig. 4B). The very tips of the free vein-endings are occupied by tracheids which are clavate. The marginal terminations of the veinlets are characteristically spread out or broom-shaped (Fig. 4C).

S. laurina (Retz.) Wall. ex G. Don, Syst. 4: 3. 1837. *Myrtus laurina* Retz. Obs. Bot. 26. 1786. *Symplocos spicata* Roxb. Fl. Ind. 2 ed. 541. 1832; Clarke in Fl. Brit. Ind. 3: 573. 1882.

Taxonomy: Small trees 7-10 m tall. Leaves broadly lanceolate, c 12.50 × 5 cm, acute, serrulate, lamina base cuneate, glabrous; petiole c 6 mm long. Spikes branched, rusty pubescent or even glabrous; flowers sessile, sweet smelling; bracts and bracteoles overlapping the calyx-tube; calyx teeth minute, c 1 mm; corolla c 8 mm; stamens about 40 to 50. Fruits 5 mm across, globose, ribbed; calyx-rim as wide as fruit.

Anatomy: *Petiole*—The median vascular bundle is crescent-shaped and gives branches from the proximal end, and these in turn divide further (Fig. 5A). There is no sclerenchyma in the cortex.

Venation: The ratio between the veins of 3rd and last category is 2: 1 (Fig. 11). The bundle sheath is parenchymatous. The ultimate veinlets branch profusely like staghorn, and the very tips are occupied by tracheids which are clavate and some are dilated (Fig. 5B). The marginal veinlets at the serrulations are fusiform.

S. phyllocalyx Clarke in Fl. Brit. Ind. 3: 575. 1882. *S. japonica* A. DC. in DC. Prodr. 7: 255. 1844.

Taxonomy: Trees c 5-9 m tall, sometimes shrubs. Leaves oblong c 10 × 3.75 cm, acuminate; lamina base cuneate, serrulate, coriaceous, glabrous; petiole c 8 mm long. Spikes very short, pilose; bracts glabrous; calyx-tube c 3 mm, teeth ciliate along the margin; corolla c 4 mm; stamens indefinite. Fruits c 1.25 cm, ellipsoid; calyx-teeth unaltered in size in fruit.

Anatomy: *Petiole*—The median vascular bundle is crescent-shaped at the proximal end but gradually gets flattened at the distal end, giving no branches (Fig. 6A). Sclerenchyma is absent in the cortical region.

Venation: The ratio between the veins of 3rd and last category is 2.5: 1. The bundle sheath is parenchymatous but the cells are very thin-walled and hence the

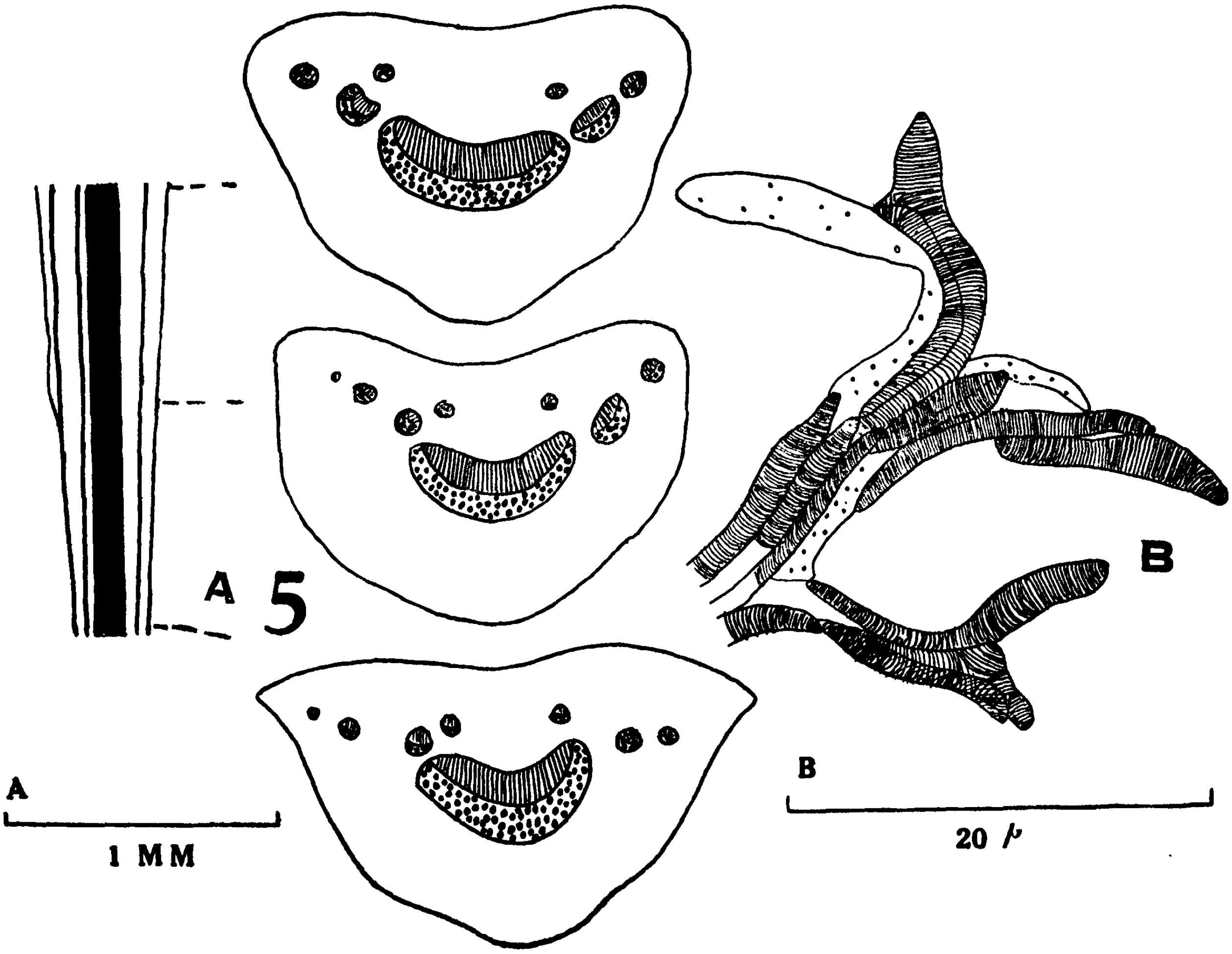


Fig. 5. *S. laurina*
(A-Trans. sections of the petiole; B-Vein endings with the bundle sheath)

sheath is very faint. The free vein endings are profusely branched and the very tips are made of 3-5 short elements. The marginal free ends along the serrulations supplying the glands are broom-shaped (Fig. 6C).

S. pyrifolia Wall. ex G. Don, Gen. Hist. 4: 3. 1837; Clarke in Fl. Brit. Ind. 3: 579. 1882.

Taxonomy: Small trees resembling *S. sumantia.* Leaves elliptic-lanceolate, c 15 × 4.5 cm, acuminate, minutely toothed, glabrous; petiole c 8 mm long. Flowers in racemes; calyx-teeth densely silky. Fruits cylindrical-ellipsoid, blue.

Anatomy: *Petiole*—The median vascular bundle is in the form of a flattened arc and does not give branches (Fig. 7A).

Venation: The ratio between the veins of 3rd and last category is 2.5:1. The bundle sheath is fibrous (Fig. 7B). The very tips of the veinlets are made of elongate-clavate elements. The marginal veins to the serrulations gradually taper and end there (Fig. 7C).

S. racemosa Roxb. Fl. Ind. 2 ed. 539. 1824; Clarke in Fl. Brit. Ind. 3: 576. 1882.

Taxonomy: Small trees c 4.5-7 m tall, frequently shrubs. Leaves oblong, cuneate at both ends, c 12.50 × 4 cm, obscurely crenate, coriaceous; petiole c 8 mm long. Racemes hairy; bracts ovate, hairy; calyx-tube glabrous but teeth minutely pubescent; corolla c 4 mm; stamens indefinite. Fruit

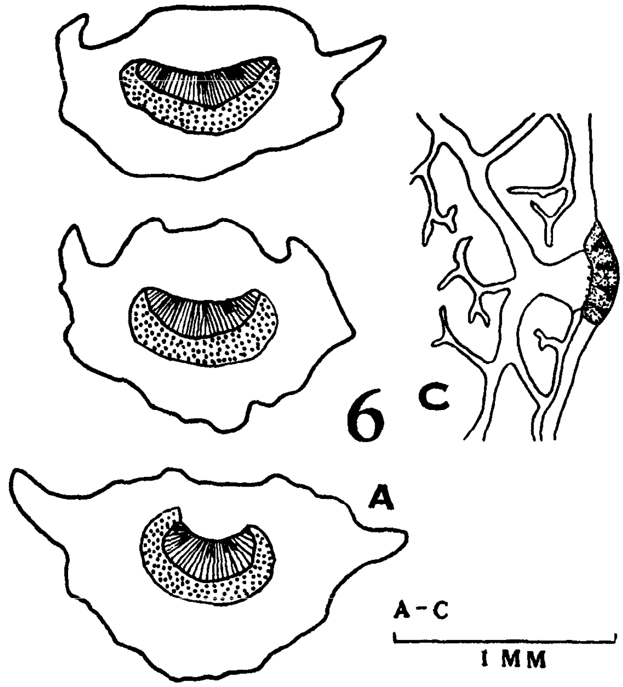


Fig. 6. *S. phyllocalyx*
(A-Trans. sections of the petiole ; C-Marginal serration)

c 1.2 cm long, subcylindric; calyx-rim as wide as fruit; teeth erect.

Anatomy: *Petiole*—The median vascular bundle is in the form of a flattened arc, and gives no branches. Sclerenchyma is absent in the cortical region.

Venation: The ratio of the veins of 3rd and last category is 6: 1. The bundle sheath is parenchymatous. The terminal elements of the very tips are elongate-clavate. The marginal free ends along the serrulations are broom-shaped.

S. ramosissima Wall. ex G. Don, Gen. Hist. 4:3. 1837; Clarke in Fl. Brit. Ind. 3: 577. 1882.

Taxonomy: Shrubs sometimes trees c 10-12 m tall. Leaves oblong-lanceolate, c 10 × 3.5 cm, sometimes longer, acuminate, lamina base cuneate; petiole c 6 mm long. Racemes few flowered; bracts deciduous; bracteoles closely adpressed to the calyx-tube; corolla c 3-4 mm; stamens 20. Fruits c 6 mm or longer, ellipsoid; calyx-rim narrower than fruit.

Anatomy: *Petiole*—The petiole has a deep groove on the adaxial surface. The median vascular bundle is crescent-shaped, giving no branches (Fig. 8A).

Venation: The ratio between the veins of 3rd and last category of veinlets is 8: 1 (Fig. 13). There is a double sheath consisting of a sclerenchymatous sheath covered by a parenchymatous sheath. The terminal elements of the very tips are elongate-clavate. The marginal free ends of the veinlets are broom-shaped (Fig. 8C).

S. sumantia Buch.-Ham. ex D. Don, Prodr. Fl. Nep. 144. 1825; Clarke in Fl. Brit. Ind. 3: 578. 1882.

Taxonomy: Small trees. Leaves oblong-lanceolate, c 12 × 3.25 cm, acuminate, lamina base cuneate, crenulate, coriaceous; petiole c 6 mm long. Racemes hirsute; bracts and bracteoles villous, caducous; calyx-tube glabrous; corolla c 4 mm; stamens in 5 bundles, each with 7-8 stamens. Fruits c 9 mm long, ovoid-cylindric; calyx-rim as wide as the upper part of the fruit.

Anatomy: *Petiole*—The median vascular bundle is in the form of a flattened arc. There are no branches arising from the median bundle.

Venation: The ratio between the veins of 3rd and last category of veinlets is 3: 1 (Fig. 15). The bundle sheath is sclerenchymatous. The terminal elements are elongate-clavate. The submarginal free veinlets taper gradually and terminate in the marginal serrulations.

S. theaefolia D. Don, Prodr. Fl. Nep. 145. 1825; Clarke in Fl. Brit. Ind. 3: 575. 1882.

Taxonomy: Trees, c 10-15 m tall. Leaves oblong, c 10 × 4 cm, acuminate, lamina base cuneate, obscurely serrulate, glabrous; petiole c 6 mm long. Spikes branched, minute-

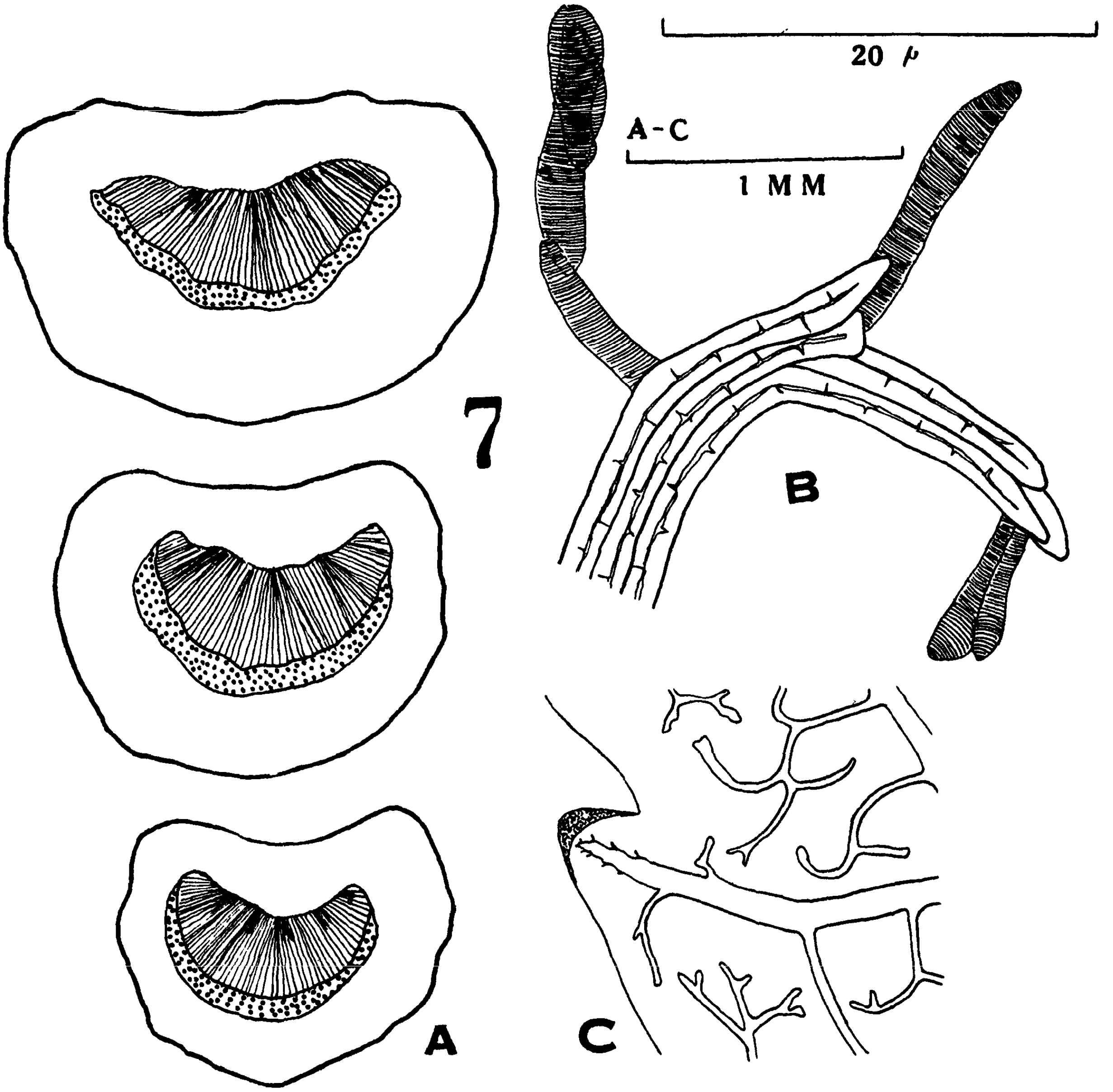


Fig. 7. *S. pyrifolia*
(A-Trans. sections of the petiole ; B-Vein endings with the bundle sheath ; C-Marginal serration)

ly pubescent ; bracts and bracteoles obtuse ; calyx-tube glabrous ; teeth ciliate ; corolla 4-3 mm ; stamens about 20. Fruits 8 mm, ellipsoid ; calyx-teeth inconspicuous.

Anatomy: *Petiole*—The median vascular bundle is in the form of a flattened arc and gives no branches (Fig. 9A).

Venation: The ratio between the vein of 3rd and last category is 2 : 1. The veins and veinlets are covered by a parenchymatous bundle sheath. The terminal elements of the free veinlets are elongate-clavate. The submarginal free vein endings to the serrulations are fusiform (Fig. 9C).

DISCUSSION

According to Petit (1887) "Coupe caractéristique" is the transverse section through the distal end of the petiole, and he has based his observations largely on such single

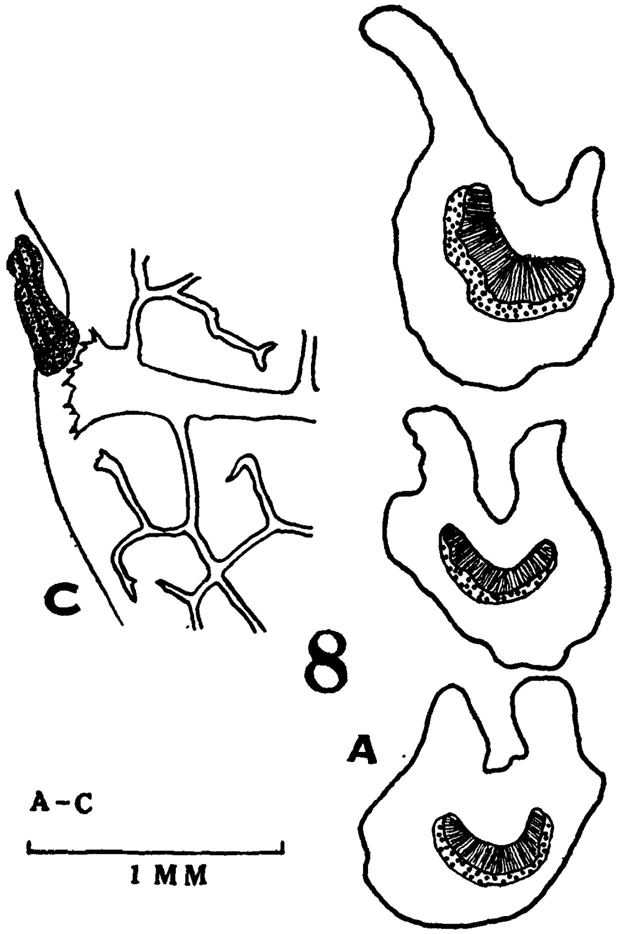


Fig. 8. *S. ramosissima*
(A-Trans. sections of the petiole ; C-Marginal serration)

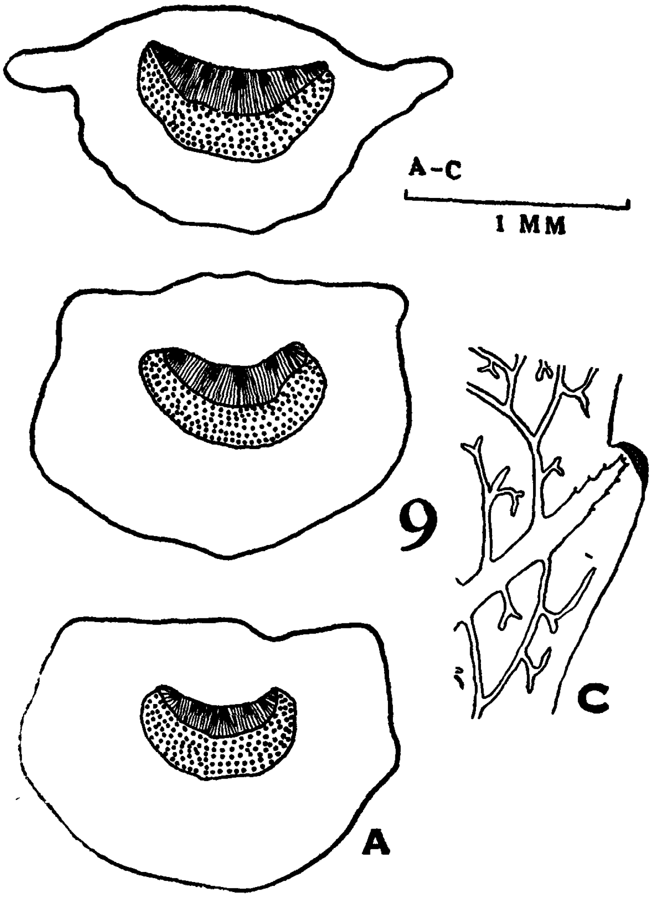
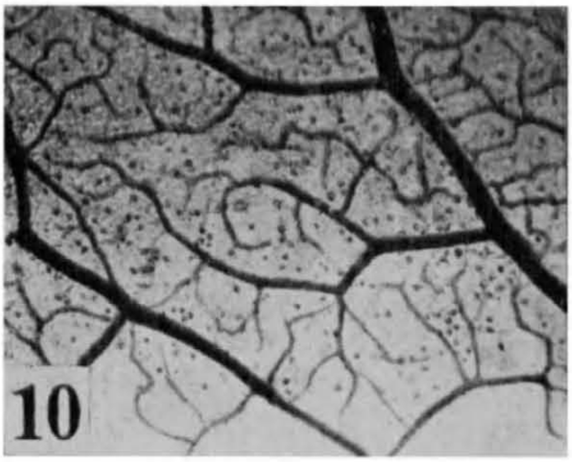
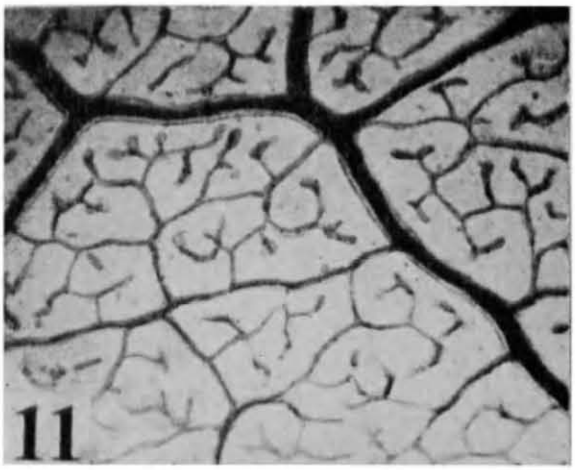


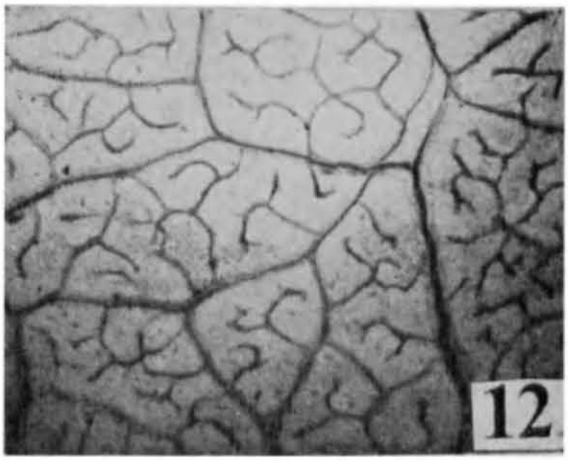
Fig. 9. *S. theaefolia*
(A-Trans. sections of the petiole ; C-Marginal serration)

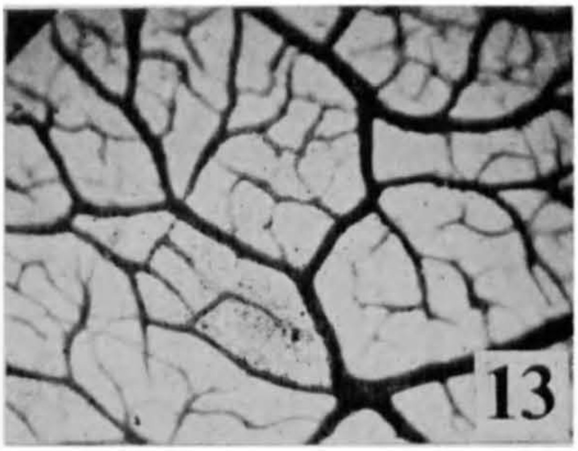
sections. Metcalf and Chalk (1950) suggest that further investigation of the vascular structures of the petiole may yield patterns of diagnostic value in certain families and genera; they have also followed Petit in basing their observations on "Coupe caracteristique". According to Howard (1962) the vascular structure from the middle of the petiole appears to be the most useful at the generic level and in a few cases the petiole vascular pattern is of use at the specific level. Decker (1967) views that the variations in the vascularisation of the petiole of Luxemburghieae (Ochnaceae) appears to be significant in determining the phylogeny of the tribe. Dickison (1969) finds that the petiole anatomy of the Dilleniaceae shows considerable variations both between and within the genera, and according to him the diversity present at the specific level indicates that the anatomy of the petiole may be of taxonomic significance in the family. In the present study, although limited to only eleven species occurring in Nepal, diversity in the petiole anatomy has been observed at the specific level. Sections of the petiole have been studied at the proximal, middle and distal ends and the structural variations of the vascular bundles checked at these different levels. This lends support to the view that petiole anatomy can be of significance in atleast these eleven species of *Symplocos* as in Dilleniaceae.

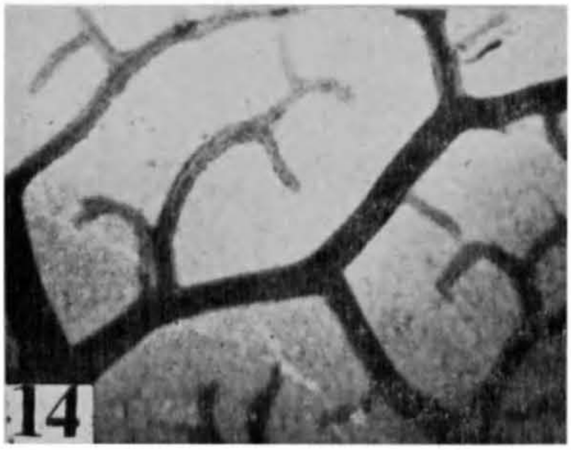
It is of interest to find sclerenchymatous bundle sheath in *Symplocos caudata, S. pyrifolia, S. ramosissima* and *S. sumantia,* as in certain other

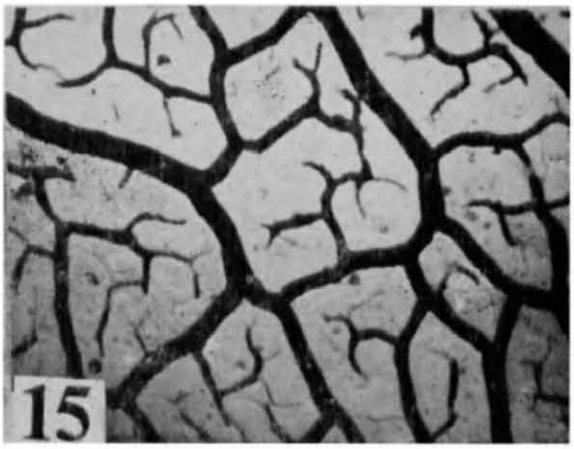












Figs. 10-15 : Minor venation patterns in some species of *Symplocos*. 10. *S. caudata*. 11. *S. laurina*. 12. *S. crataegoides*. 13. *S. ramosissima*. 14. *S. dryophila* 15. *S. sumantia*.

families like Winteraceae and Melastomataceae (see Esau, 1965) and in certain Dilleniaceae particularly *Hibbertia banksii* where an interrupted sclerenchymatous sheath is present in the leaves (Dickison, 1969). According to Kasapligil (1964) in *Corylus avellana* and *C. maxima* the ultimate areoles may or may not be provided with a vein ending, while in *C. colurna* most of them are provided with freely terminating veins. Banerji and Das (1972) have observed that in *Acer oblongum* the areoles do not have free terminations of the veinlets, while all the other Indian species of *Acer*, studied by them have free vein terminations. Yaltirik (1971) who has similar observations on the acers of Turkey, uses this as a character for the separation of species and varieties of the Turkish acers. In all the species of *Symplocos* studied in the present investigation the areoles have free vein endings. Dickison (*loc. cit.*) makes distinction between genera and species on the basis of the diameter of the veins and veinlets; thus according to him, some genera of Dilleniaceae have very slender veins. Such distinction can also be made between the eleven species of *Symplocos*.

It may be pertinent to add that according to Carlquist (1959) minor venation patterns provide taxonomic and evolutionary clues in certain Hawaiian Composits. The present study on a limited number of species of *Symplocos* supports the views of Carlquist.

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"SYMPLOCACEAE OF THE WORLD" by Nooteboom has appeared on 1976. If petiole anatomy is to be relied upon to be useful at the specific level, even of a few species, as has been advocated by Howard (1962). Decker (1967), Schofield (1968) and Jansen & Baas (1973), we are disinclined to accept some of the nomenclatural changes effected by Nooteboom. There are clear differences in the bundle sheaths of *S. phyllocalyx* and *S. theaefolia*, and further the veinlets along the margin are also different in the two species.—AUTHOR.

The nomenclatural changes effected by Nooteboom in the above mongraph are as follows : 1. *Symplocos crategoides* Buch.-Ham. ex D. Don has been reduced to **S. paniculata** (Miq.) Thunb. 2. *S. laurina* (Retz.) Wall. ex G. Don has been reduced to **S. cochinchinensis** (Lour.) Moore 3. *S. phyllocalyx* Clarke and *S. theaefolia* D. Don have both been reduced to **S. lucida** (Thunb.) S. & Z.—EDITOR